REMARKS

Claims 12-22 and 24-28 are pending. Claims 12-15 and 24-28 are currently under examination. Claims 16-22 are withdrawn from consideration. Claims 12-14, 24, and 25 have been amended. This amendment was suggested by the Examiner in the current Office Action at the bottom of page 4. Support in the specification is found in the recitation of art recognized voltage-gated sodium channel inhibitors such as those found on page 1, line 35 through page 4, line 28.

Accordingly, these amendments do not raise an issue of new matter and entry thereof is respectfully requested.

Rejection Under 35 U.S.C. §112, first paragraph

The rejection of claims 12, 14, and 23-28 under 35 U.S.C. §112, first paragraph is respectfully traversed.

While Applicants disagree with the Examiner's underlying reasoning for this rejection, in an effort to move prosecution forward, Applicants have made the Examiner's suggested amendments to the claims replacing "a sodium channel-inhibiting substance" with "a voltage-gated sodium channel inhibitor." Applicants respectfully request withdrawal of this rejection. Applicants note that claim 23 has been canceled rendering its rejection moot.

Rejection Under 35 U.S.C. §103

The rejection of claims 12, 13, and 23-28 under 35 U.S.C. §103(a) as being unpatentable over Rundfeldt et al. (U.S. 6,117,900) in view of Cai et al. (U.S. 6,281,211) and in further view of art of record in Applicant's specification is respectfully traversed (Office Action at item 4, pages 5-8).

Amended independent claim 12 recites a method of treating neuralgia pain or neuropathic pain, in a mammal, comprising administering to a patient in need thereof a therapeutically effective amount of a combination of retigabine or a therapeutically utilizable salt thereof with a voltage-gated sodium channel inhibitor or a therapeutically utilizable salt thereof.

The Examiner alleges that Rundfeldt et al. teach the use of retigabine for the treatment of neuropathic pain in an animal and that Cai et al. teach sodium channel blockers such as riluzole, lidocaine, propafenone, and semicarbazone derivatives for the treatment of neuropathic pain in mammals, including humans. Furthermore, the Examiner alleges that the art of record in the specification teaches the use of sodium channel inhibitors or tolperisone to normalize muscle tone.

The Examiner concludes that it would be obvious to combine retigabine as taught by Rundfeldt et al. with sodium channel blockers, such as lidocaine, propafenone and riluzole, as taught by Cai et al. because it is allegedly obvious to combine two compositions each of which is taught in the prior art to be useful for the same purpose.

Applicants wish to point out that Cai et al. appear to teach that carbamazepine, lidocaine, and phenytoin have been used to treat neuropathic pain.

> "In addition to the above-mentioned clinical uses, carbamazepine, lidocaine, and phenytoin are occasionally used to treat neuropathic pain," (col.1, lines 49-52)

However, Cai et al. appears to teach that propafenone is an antiarrhythmic but is silent with respect to its use to treat neuropathic pain. Likewise, Cai et al. appears to teach that sodium channel inhibitor riluzole is useful for the treatment of ALS, but does not address its use to treat neuropathic pain:

> "Another example of clinical use of a Na^+ channel blocker is riluzole. This drug has been shown to prolong survival in a subset of patients with ALS (Bensimm et al., *New Engl. J. Med.* 330:585-591 (1994)) and has subsequently been approved by the FDA for the treatment of ALS." (col. 1, lines 44-48)

It appears the Examiner may have erred in characterizing Cai et al. as providing a general teaching that <u>any</u> sodium channel blocker is useful for treating neuropathic pain. To further support this point, Applicants submit Exhibit A: Galer et al., "Lack of efficacy of riluzole in the treatment of peripheral neuropathic pain conditions." *Neurology* 55:971-975 (2000), as evidence that sodium channel blocker riluzole, in particular, would not be expected to be useful for the treatment of neuropathic pain. One skilled in the art, aware of the teachings of Galer et al. would not come to the conclusion that 1) <u>any</u> sodium blocker would be effective to treat neuropathic pain or 2) that <u>any</u> sodium channel blocker would be expected to have an added benefit when

used in conjunction with retigabine. Indeed, Galer et al. specifically teach away from a combination of riluzole and retigabine to treat neuropathic pain because riluzole has been found to be ineffective for this purpose. Applicants, therefore respectfully traverse the Examiner's position that sodium channel blockers share the same purpose as retigabine with respect to the treatment of neuropathic pain.

Similarly, with respect to tolperisone, Applicants wish to point out that this compound and its analogues are disclosed as muscle relaxants for the treatment of increased muscle tone. Increased muscle tone is not the same indication as neuropathic pain and therefore tolperisone and retigabine are not taught in the art to be useful for the same purpose.

Furthermore, Applicants respectfully disagree that two drugs indicated for treatment of neuropathic pain renders obvious their combination. The combination of lidocaine, or any other sodium channel inhibitor, with retigabine, a potassium channel opener, is not obvious because of the complexity of cellular mechanisms, such as the sodium-potassium ATPase pump, dedicated to the balance of sodium and potassium ions inside and outside of cells. The concentration differences between potassium and sodium ions maintained by this pump help create a membrane potential that is important for heart function, nerve impulse transmission, muscle contraction, and maintaining cellular osmotic pressure (Applicants submit Exhibit B: Alberts et al. Molecular Biology of the Cell, 3rd ed. 1994 Garland Publishing, Inc. New York & London, 513-516). The interplay and distribution of such pumps, in conjunction with voltage-gated sodium and potassium channels translates to a level of unpredictability as to whether each drug would have the same impact used together as they would have when used separately. Thus, one skilled in the art is not instilled with a reasonable expectation of success that the drug combination would have synergistic or even simple additive beneficial effects.

In establishing a *prima facie* case of obviousness through the combination of the teachings, the Examiner must show that there was a motivation or rational underpinning for combining the references and that there was a reasonable expectation of success. Applicants assert that the Examiner was deficient in showing a motivation or rational underpinning to combine the use of retigabine with voltage-gated sodium channel inhibitors, because sodium channel inhibitors do not generally have the property of being useful for the treatment of neuropathic pain. Furthermore, Applicants assert that the Examiner has failed to demonstrate

that one skilled in the art would have a reasonable expectation of success in combining retigabine with a voltage-gated sodium channel inhibitor because of competing mechanistic pathways that regulate intracellular and extracellular concentrations of potassium and sodium.

In light of the above, Applicants submit that independent claim 12 is patentable over Rundfeldt et al. (U.S. 6,117,900) in view of Cai et al. (U.S. 6,281,211) and in further view of art of record in Applicant's specification. Claims 13, 24-26, and 28 depend from claim 12 and are patentable for at least the same reasons. Applicants respectfully note that the Examiner has not explicitly rejected claim 15 under this item, but have rejected claim 27. Applicants submit that claim 27 is patentable for at least the same reasons as claim 15, which is detailed in the discussion that follows. Applicants respectfully request withdrawal of this rejection. Applicants note claim 23 has been canceled rendering its rejection moot.

The rejection of claims 14 and 15 under 35 U.S.C. §103(a) as being unpatentable over Rundfeldt et al. (U.S. 6,117,900) in view of Cai et al. (U.S. 6,281,211) and in further view of art of record in Applicant's specification is respectfully traversed (Office Action at item 5, page 8).

Claim 14 is patentable for at least the same reasons as claim 12 from which it depends, as described above. Applicants respectfully request withdrawal of this rejection.

Claim 15 recites a method of treating neuralgia pain or neuropathic pain, in a mammal, comprising administering to a patient in need thereof a therapeutically effective amount of a combination of retigabine with tolperisone, eperisone, silperisone, or tolperisone analog, or a pharmaceutically utilizable salt thereof.

The Examiner alleges that tolperisone is a sodium channel blocker similar to lidocaine and would be expected to behave in a similar manner. Applicants have established that the property of being a sodium channel blocker does not instill a compound with the property of being useful for the treatment of neuropathic pain, as exemplified by riluzole discussed above. Applicants also respectfully point out that tolperisone has not been indicated for the treatment of neuropathic pain, rather it is indicated for the treatment of increased muscle tone.

One skilled in the art would not expect lidocaine and tolperisone to necessarily behave in a similar manner because while there are some similarities, there are also differences. In this

regard, the Examiner's attention is respectfully drawn to the specification as filed at page 2, line 39 through page 3, line 2: "As compared with lidocaine, a local anesthetic, the substance [tolperisone] has less of a blocking effect on transmission in the A fibers." and page 3, lines 23-25: "It is probable that the mechanism of action of tolperisone in this connection differs somewhat from that of lidocaine."

Even assuming *arguendo* that tolperisone and lidocaine were identical, Applicants draw from the discussion above regarding the lack of expectation of success in the combination of tolperisone, a sodium channel inhibitor, with retigabine, a potassium channel opener. Applicants respectfully request withdrawal of this rejection.

Entry of the proposed amendments is respectfully submitted to be proper because the amendments are believed to place the claims in condition for allowance. In light of the amendments and remarks herein, Applicants submit that the claims are now in condition for allowance and respectfully request a notice to this effect. The Examiner is invited to call the undersigned agent if there are any questions.

To the extent necessary, a petition for an extension of time under 37 C.F.R. 1.136 is hereby made. Please charge any shortage in fees due in connection with the filing of this paper, including extension of time fees, to Deposit Account 502624 and please credit any excess fees to such deposit account.

Respectfully submitted,

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Lack of efficacy of riluzole in the treatment of peripheral neuropathic pain conditions

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Article abstract—*Objective:* To assess the efficacy, tolerability, and safety of riluzole in the treatment of peripheral neuropathic pain conditions. *Background:* Both basic and clinical research has demonstrated that drugs with sodium channel and NMDA antagonism can be effective in alleviating neuropathic pain. Riluzole, a drug currently used for treatment of ALS, possesses these properties. It was hypothesized that riluzole would be effective in reducing the pain in subjects with peripheral neuropathic pain. *Methods:* Two randomized, placebo-controlled, crossover studies were performed at two sites. Study 1 compared 100 mg/day of riluzole (the currently recommended dosage for treatment of ALS) versus placebo, and Study 2 compared 200 mg/day of riluzole versus placebo. Each treatment phase (both studies) was 2 weeks long, separated by 2-week wash-out periods. Outcome measures included change in the score on a 100-mm pain intensity visual analog scale, the Neuropathic Pain Scale, allodynia, hyperalgesia, and preference for study treatment phase. *Results:* Twenty-two subjects completed Study 1, and 21 subjects completed Study 2. Four subjects (two from each study) discontinued the study because of intolerable side effects. No statistical difference was found for any study outcome measure between riluzole and placebo for either study. In Study 1, pain intensity was more likely to increase than decrease with riluzole (mean treatment difference 8.7 mm; 95% CI −19.5 to +2.1 mm). In Study 2, very slight pain reduction was observed with riluzole compared with placebo (mean treatment difference 1.4 mm; 95% CI −5.1 to +8.0 mm). In both studies, the majority of subjects chose “no change” in pain on the category relief scale after placebo and riluzole treatment phases. On study completion, no treatment preference was reported by 76% of the subjects in Study 1 and by 61% of the subjects in Study 2. *Conclusions:* Doses of riluzole at (100 mg) or above (200 mg) those used for the treatment of ALS were not effective in alleviating peripheral neuropathic pain.

NEUROLOGY 2000;55:971–975

Animal models have demonstrated several potential pain mechanisms underlying the generation and maintenance of neuropathic pain.[1] These mechanisms include ectopic impulse generation associated with an upregulation of sodium channels[2] and central sensitization dependent on excitatory amino acid receptor activity.[3] In fact, several drug classes that have been shown in randomized controlled trials to be of benefit in neuropathic pain are believed to act, at least in part, via sodium channel blockade; among these are tricyclic antidepressants such as amitriptyline, anticonvulsants such as carbamazepine and phenytoin, and local anesthetic agents such as lidocaine and mexiletine.[4] Studies have reported an improvement of animal pain behavioral measures after administration of NMDA antagonists such as dextromethorphan and MK-801.[5] Additionally, a small controlled clinical study has observed efficacy of dextromethorphan.[6]

Thus, a drug that blocks sodium channels and has

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Supported by research grants from Rhone–Poulenc Rorer to the University of Washington School of Medicine and the University of California San Francisco School of Medicine.

Received December 16, 1999. Accepted in final form June 13, 2000.

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Exhibit A

NMDA antagonistic actions would appear to be a good candidate for relieving neuropathic pain of peripheral origin. The drug riluzole has such properties, inactivating voltage-dependent sodium channels and depressing glutaminergic neurotransmission.[7] Riluzole has been shown to have antiepileptic activity in animal models.[8] Riluzole is currently approved in the United States for the palliative treatment of ALS. Therefore, based on the known pharmacologic properties of the drug, a randomized controlled trial was performed assessing the efficacy and tolerability of riluzole for treatment of peripheral neuropathic pain.

Methods. *Study design and subjects.* Two prospective, randomized, crossover, double-blind clinical trials of identical design were performed. Study 1 compared placebo with 100 mg/day of riluzole (the recommended daily dose for the treatment of ALS), both administered for a period of 2 weeks. Study 2 compared placebo with 200 mg/day of riluzole, both for 2 weeks. These studies were conducted from June 10, 1997, to January 30, 1998, at the University of Washington at Seattle and the University of California at San Francisco (UCSF). The research protocols and informed consents were approved by institutional review boards at each site.

The entry criteria for both studies were identical. Adults at least 18 years of age were eligible if they were in stable general health, had no contraindications to riluzole therapy, and had a diagnosis of chronic peripheral neuropathic pain due to peripheral neuropathy or focal peripheral nerve injury of least 3 months' duration. Subjects were excluded from the study according to the following criteria: existence of another pain problem of equal or greater severity; history of liver disease; history of alcohol abuse according to criteria from the *Diagnostic and Statistical Manual of Mental Disorders,* 4th edition[9]; use of antidepressants, anticonvulsants, or mexiletine for the treatment of pain 7 days before entry into the study; alanine aminotransferase, aspartate aminotransferase, or bilirubin in the abnormal range or a low white blood cell count in the blood sample taken during the screening visit; psychiatric or seizure disorder requiring the continued use of a prohibited antidepressant or anticonvulsant drugs; and, in women, pregnancy, lactation, or nonadherence to adequate birth control methods.

Study procedures in both Study 1 and Study 2 were identical; the only difference between the two studies was the dosage of the study drug. In Study 1, subjects were instructed to take one capsule twice per day during each treatment phase; active capsules contained 50 mg of riluzole, for a total of 100 mg/day. Subjects enrolled in Study 2 took two capsules twice per day, for a total of 200 mg/day; active capsules were identical in appearance to placebo capsules. Subjects who participated in the 100-mg/day dose trial were not enrolled in the 200-mg/day dose trial.

After informed written consent was obtained at the screening visit, eligible subjects were oriented to all study procedures and instructed on the completion of daily pain diaries, pain intensity ratings, and recording of medication usage. Subjects underwent physical, neurologic, and sensory examinations. Allodynia to stroking with a foam paintbrush and hyperalgesia to repetitive gentle tapping with a safety pin in the area of greatest pain were separately rated by the examiner on a 0 to 3 scale as 0 = none, 1 = mild, 2 = moderate, and 3 = severe. Demographic information and medical histories were obtained. Blood samples were obtained for routine hematology and chemistry studies, and subjects completed a written 100-mm Pain Visual Analogue Scale (Pain VAS; with 0 = no pain and 100 = worst pain imaginable) and the Neuropathic Pain Scale (NPS), a series of 10 scales rating different symptoms of neuropathic pain on 0 to 10 Likert scales.

Subjects initially entered a 1-week baseline data collection period followed by a 2-week treatment phase (treatment phase A), a 2-week wash-out phase, a second 2-week treatment phase (treatment phase B), and a final 1-week wash-out phase. In each study, subjects were seen for six scheduled visits: an initial screening visit (visit 1), start of treatment phase A (visit 2), end of treatment phase A (visit 3), end of wash-out A/start of treatment phase B (visit 4), end of treatment phase B (visit 5), and end of wash-out B (visit 6).

Subjects continued into treatment phase A (visit 3) if they had pain of at least moderate severity (defined as 30 mm or more on the 100-mm daily diary Pain VAS) during the baseline week. Neurologic and sensory examinations were updated, and the Pain VAS, a six-item Category Pain Relief Scale (CRS; with 0 = pain worse, 1 = no relief, 2 = slight relief, 3 = moderate relief, 4 = a lot of relief, 5 = complete relief), and NPS were completed. Subjects were randomly assigned to either active drug or placebo capsules. After 2 weeks of therapy, subjects returned to the clinic (visit 3). Physical, neurologic, and sensory examinations were updated; blood samples were obtained for routine hematology and chemistry studies; pain diaries and study medication were collected; medication use adverse events were recorded; and subjects again completed the Pain VAS, CRS, and NPS. After visit 3, subjects were sent home without any study drug for 2 weeks. Subjects then returned for visit 4 and were crossed over to the opposite treatment arm (treatment phase B) if their pain intensity levels had returned to baseline. After 2 weeks of treatment

Table 1 *Demographic and baseline characteristics for subjects in Study 1 and Study 2*

Characteristics	Study 1 100 mg/d (n = 22)	Study 2 200 mg/d (n = 21)
Sex		
M/F	12/10	13/8
Age, y, median (range)	66 (33–87)	64 (35–93)
Race, n		
White	19	17
Others	3	4
Pain diagnosis, n		
PHN	13	9
DN	1	1
PN	8	11
Baseline average daily pain score, mean (SD)	69.6 (17.4)	66.9 (17.6)

PHN = postherpetic neuralgia; DN = diabetic polyneuropathy; PN = peripheral neuropathy other than diabetic neuropathy.

phase B, subjects returned for visit 5. They were then sent home for a 1-week wash-out period and returned for a final visit (visit 6). At visit 6, the subject was asked to identify a preferred treatment phase.

In addition to study visits, subjects were contacted by phone once per week to assess adherence to the study drug administration schedule, daily diary completion, and monitoring of study-related adverse events or complications.

Randomization, data collection, and statistical analyses. A stratified block randomization scheme was used to assign subjects to either riluzole–placebo or placebo–riluzole sequences. Riluzole and placebo capsules were identical in appearance and were packaged in subject-specific bottles according to the randomization schedule. During both Study 1 and Study 2, subjects were instructed to take one capsule twice per day and were provided with a written schedule of study medication administration dose and time guidelines.

Statistical analyses were performed separately for each trial on the efficacy-evaluable populations, which consisted of those subjects who were adherent to the study protocols and completed the low-dose (n = 22) and high-dose (n = 21) studies. Primary outcome measures were comparisons of the change in pain scores using visit data (i.e., ratings of pain at the time of the clinic visit) from the start of the treatment arm to the end of the treatment arm for the active drug and placebo phases in each study. The change in pain was calculated as the difference between pain at the start and pain at the end of the treatment arm. Repeated measures of analysis of variance (ANOVA) were used to test for sequence (carryover), drug, and time effects. Secondary outcome measures included the change in average daily Pain VAS scores. Change scores were calculated as the difference between average daily Pain VAS scores from the week before the start of treatment arm to the second week of the treatment arm for active drug and placebo phases in each trial. A repeated-measures ANOVA was used to determine significance. Additional secondary outcome measures included change in CRS ratings, allodynia and hyperalgesia ratings, and NPS scores. A repeated-measures ANOVA was used to compare sequence effects and CRS scores at the last visit of the treatment arm for the active drug versus placebo phases. Change scores for allodynia ratings, hyperalgesia ratings, and the NPS scale were calculated in the same fashion as the primary outcome measure and were analyzed using Wilcoxon's Signed Ranks Test. All p values reported are two-tailed.

Results. A total of 55 subjects were enrolled in the trials: 36 at the UCSF Pain Clinical Research Center and 19 at the University of Washington Pain Clinical Research Center. Forty-three subjects completed the trials. In Study 1, 13 completing subjects had postherpetic neuralgia, eight had a peripheral neuropathy other than diabetic neuropathy, and one had diabetic polyneuropathy. In Study 2, nine subjects had postherpetic neuralgia, 11 had a peripheral neuropathy other than diabetic neuropathy, and one had diabetic polyneuropathy. Baseline characteristics of the subjects in both trials are shown in table 1.

Study 1. In the 100-mg/day dose study, 29 subjects were screened and randomized at the baseline visit. Twenty-two (76%) completed the trial, and seven (24%) discontinued. Of the subjects who discontinued, three withdrew during the placebo phase, and four dropped out

***Table 2** Summary of primary and secondary outcome measures*

Measures	Pretreatment	End-of-treatment	Change from pretreatment to end of treatment
Study 1: low-dose riluzole, n = 22			
Visit data Pain VAS score			
Placebo	69.4 (18.9)	68.6 (26.1)	−0.7 (20.8)*
Riluzole	66.7 (21.6)	74.7 (19.5)	8.0 (13.3)
Average daily Pain VAS score			
Placebo	69.7 (17.0)	68.1 (22.6)	−1.6 (9.6)*
Riluzole	69.3 (20.1)	69.2 (20.5)	−0.1 (9.0)
Average NPS score			
Placebo	5.5 (3.0)	5.2 (1.9)	−0.2 (1.7)*
Riluzole	5.0 (1.4)	5.2 (1.4)	0.1 (1.0)
Average CRS			
Placebo	—	1.1 (0.7)*	—
Riluzole	—	1.0 (0.4)	—
Average allodynia			
Placebo	1.5 (1.1)	1.4 (1.2)	−0.1 (0.7)*
Riluzole	1.5 (1.1)	1.5 (1.3)	0.0 (0.8)
Average hyperalgesia			
Placebo	1.3 (0.7)	1.4 (0.9)	0.2 (0.5)*
Riluzole	1.3 (1.1)	1.6 (1.2)	0.3 (0.7)
Study 2: high-dose riluzole, n = 21			
Visit data Pain VAS score			
Placebo	65.6 (19.2)	64.0 (24.8)	−1.6 (12.0)*
Riluzole	65.5 (17.5)	62.5 (19.2)	−3.0 (8.4)
Average daily Pain VAS score			
Placebo	68.0 (17.9)	64.8 (22.9)	−3.2 (10.7)*
Riluzole	64.9 (17.5)	64.9 (17.2)	0.0 (6.9)
Average NPS score			
Placebo	5.3 (1.3)	5.0 (1.3)	−0.3 (0.7)*
Riluzole	4.7 (1.3)	4.2 (1.8)	−0.5 (0.8)
Average CRS			
Placebo	—	1.0 (0.9)*	—
Riluzole	—	1.4 (0.8)	—
Average allodynia			
Placebo	1.3 (1.2)	1.2 (1.2)	−0.2 (0.7)*
Riluzole	1.0 (1.2)	1.0 (1.3)	−0.1 (0.7)
Average hyperalgesia			
Placebo	1.6 (1.2)	1.6 (1.2)	0.1 (0.4)*
Riluzole	0.9 (1.0)	0.9 (1.1)	0.0 (0.3)

All values expressed as mean (SD).

* Indicates $p > 0.10$, repeated-measures analysis of variance.

VAS = Visual Analogue Scale; NPS = Neuropathic Pain Scale; CRS = Category Pain Relief Scale.

during riluzole treatment. Early withdrawal was due to inadequate pain relief (n = 5) or unacceptable side effects (n = 2) (table 2).

No significant differences between the subject groups (riluzole–placebo versus placebo–riluzole) were noted for any baseline or demographic variable. A comparison of average daily Pain VAS scores during the week before each treatment phase was also nonsignificant and demonstrated that pain intensity after the first treatment phase returned to baseline levels before the second treatment phase began.

For the primary outcome measure, a comparison of the change in treatment arm visit Pain VAS scores for placebo versus riluzole was nonsignificant (mean decrease of 0.7 mm, SD 20.8, versus mean increase of 8.0 mm, SD 13.3). No statistical differences in average daily Pain VAS scores were found comparing riluzole and placebo from the start-of-treatment evaluation to the end-of-treatment evaluation.

All analyses of other secondary outcome measures did not show significant changes. Category Pain Relief ratings during the placebo phase demonstrated that the majority (82%) reported "no change," 9% reported "worse pain," and 9% reported "moderate relief." Similarly, during the riluzole phase 82% reported "no change," 9% reported "worse pain," and 9% reported "slight relief." Sixteen (76%) subjects rated their treatment preference as "no difference," three preferred the placebo phase, and two preferred the riluzole phase.

Study 2. In the high-dose trial, 26 subjects were screened and randomized at the baseline visit. Twenty-one (81%) completed the trial; five (19%) discontinued. Of those who discontinued, two withdrew during the placebo phase and three during the active drug phase. Early withdrawal was due to inadequate pain relief (n = 3) or unacceptable side effects (n = 2). No significant findings were noted for any baseline or demographic variables (see table 2).

Average Pain VAS scores using visit data for the start of placebo were 65.6 mm (SD 19.2) versus 65.5 mm (SD 17.5) for the riluzole treatment phases. A comparison of baseline week average daily Pain VAS scores before each treatment phase did not show a significant change and demonstrated that pain levels after the first treatment phase returned to baseline levels. No significant difference was found for the primary outcome measure, change in Pain VAS scores. All analyses of secondary variables revealed no significant difference between riluzole and placebo. The majority of subjects (52% placebo, 57% riluzole) rated end-of-treatment Category Pain Relief as "no change." The majority of high-dose subjects (61%) rated treatment preference as "no difference"; two preferred placebo and six preferred riluzole.

Discussion. Riluzole failed to reduce chronic neuropathic pain of peripheral nervous system origin at 100 mg/day (the recommended dose to treat ALS) and at 200 mg/day in this pair of randomized, double-blind, placebo-controlled, crossover studies. Comparing the two doses, no differences in effect were seen. All diagnostic groups failed to respond. Because riluzole inhibits voltage-dependent sodium channels, inhibits release of glutamic acid, and blocks excitatory amino acid receptors, we are somewhat surprised by the lack of efficacy.[7] Drugs with similar mechanisms of action have been shown to be effective in animal models of neuropathic pain[1-3] and in clinical studies on a variety of peripheral neuropathic pain disorders.[4,6] To our knowledge, no published animal studies assess the efficacy of riluzole in models of nerve injury (Medline search, "riluzole and pain," 1966–2000). A recent randomized controlled trial of riluzole in the human experimental burn-injury model reported no acute analgesic effects in normal or hyperalgesic skin.[10]

Several explanations for the observed lack of efficacy are plausible. First, perhaps the drug binds to subpopulations of sodium channels that are not involved in chronic neuropathic pain. It is now known that distinct subtypes of sodium channels are involved in the pathophysiologic alterations after nerve damage.[11] Second, riluzole may lack clinically meaningful activity at excitatory amino acid sites. Drugs that have shown efficacy in neuropathic pain conditions and that act at the NMDA site, such as ketamine and dextromethorphan, also frequently produce sedative, cognitive, and psychiatric side effects, which were not observed in this study. Third, the dosage of the drug could have been subtherapeutic. Last, the patient population studied tended to be refractory patients who had failed to respond to other commonly used adjuvant analgesics, thus perhaps making them potentially less responsive to all pharmacologic interventions.

Another interesting finding of this study was the lack of significant pain relief reported in any treatment arm of the study, active or placebo. During the placebo phase, only 9% of patients in the 100-mg dose study and no patients in the 200-mg dose study reported "moderate" or better pain relief. Although it has been asserted that many patients with neuropathic pain disorders have an unusually high placebo response rate,[12] a recent review of the published literature showed a wide variation in placebo response rates.[13] Two recent parallel design studies of gabapentin totaling nearly 400 patients reported placebo response rates (defined as a report of at least "moderate" pain relief during placebo therapy) of 12.1% in postherpetic neuralgia and 33% in diabetic neuropathy.[14,15] Why did our patients exhibit such a low placebo response rate? The majority of subjects in our study had postherpetic neuralgia, a group that in randomized controlled trials of gabapentin and dextromethorphan had lower placebo response rates than those subjects with painful diabetic neuropathy.[6,14,15] Another possibility is that these patients tended to be refractory to many prior trials of oral medication and thus their expectations of relief were low. However, it has been reported that in neuropathic pain patients, patient self-reported expectations of pain relief do not predict response to a therapeutic procedure.[16] Regardless of reason, the extremely low placebo response rates in the two studies imply that unusually high responsivity to

placebo is not an inherent characteristic of neuropathic pain patients.

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Changes in spinal cord excitability in patients affected by ulnar neuropathy

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Article abstract—*Objective:* To evaluate whether ulnar neuropathy could induce changes in spinal cord and motor cortex excitability and therefore predispose to development of focal dystonia. *Background:* A high incidence of ulnar neuropathy has been observed in patients with musician's cramp. Polygraphic electromyograph recordings in patients with entrapment of the ulnar nerve at the elbow have demonstrated long-duration bursts of co-contraction in antagonistic muscles, similar to those observed in focal dystonia. *Methods:* All control subjects and 12 patients with ulnar neuropathy underwent an electrophysiologic protocol consisting of polygraphic recordings of a repetitive tapping task of the fourth finger, assessment of reciprocal inhibition in forearm muscles, and investigation of motor cortex excitability after paired transcranial magnetic stimulation. *Results:* Eleven of 12 patients with ulnar neuropathy showed a loss of alternation and of well-formed bursts in both flexor and extensor muscles. Evaluation of reciprocal inhibition in these patients revealed a reduction in the amount of inhibition in the disynaptic and presynaptic phases. None of the patients presented with a clinically evident dystonia of the upper limb. The study of intracortical excitability after paired shocks did not reveal any difference in the amount of intracortical inhibition and facilitation compared with the control group. *Conclusions:* A peripheral nerve injury can induce a rearrangement of reciprocal inhibition circuits at the spinal cord level. These changes might predispose to the development of a focal dystonia. However, it is likely that another, yet unknown, factor is required to alter the intracortical circuits and produce a clinically evident dystonia.

NEUROLOGY 2000;55:975–978

The role of peripheral nervous system diseases and trauma in the development of dystonia remains uncertain; however, there is increasing support for the possibility of a relationship. There have been numerous reports on the association of soft tissue injury, radiculopathy, and plexopathy in patients with focal dystonia.[1-5] A high incidence of ulnar neuropathy has been observed in patients with musician's cramp. In some of these patients, the dystonic flexion of the ipsilateral little and ring fingers seems to be related

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Supported by the Italian Ministry of University and Scientific Research (MURST).
Received December 8, 1999. Accepted in final form June 12, 2000.

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MOLECULAR BIOLOGY OF
THE CELL
THIRD EDITION

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Julian Lewis • Martin Raff • Keith Roberts
James D. Watson

Garland Publishing, Inc.
New York & London

Exhibit B

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Library of Congress Cataloging-in-Publication Data
Molecular biology of the cell / Bruce Alberts . . . [et al.].—3rd ed.
p. cm.
Includes bibliographical references and index.
ISBN 0-8153-1619-4 (hard cover).—ISBN 0-8153-1620-8 (pbk.)
1. Cytology. 2. Molecular biology. I. Alberts, Bruce.
[DNLM: 1. Cells. 2. Molecular Biology. QH 581.2 M718 1994]
QH581.2.M64 1994
574.87—dc20
DNLM/DLC
for Library of Congress
93-45907
CIP

Published by Garland Publishing, Inc.
717 Fifth Avenue, New York, NY 10022

Printed in the United States of America

15 14 13 12 10 9 8 7 6

Front cover: The photograph shows a rat nerve cell in culture. It is labeled (*yellow*) with a fluorescent antibody that stains its cell body and dendritic processes. Nerve terminals (*green*) from other neurons (not visible), which have made synapses on the cell, are labeled with a different antibody. (Courtesy of Olaf Mundigl and Pietro de Camilli.)

Dedication page: Gavin Borden, late president of Garland Publishing, weathered in during his mid-1980s climb near Mount McKinley with MBoC author Bruce Alberts and famous mountaineer guide Mugs Stump (1940–1992).

Back cover: The authors, in alphabetical order, crossing Abbey Road in London on their way to lunch. Much of this third edition was written in a house just around the corner. (Photograph by Richard Olivier.)

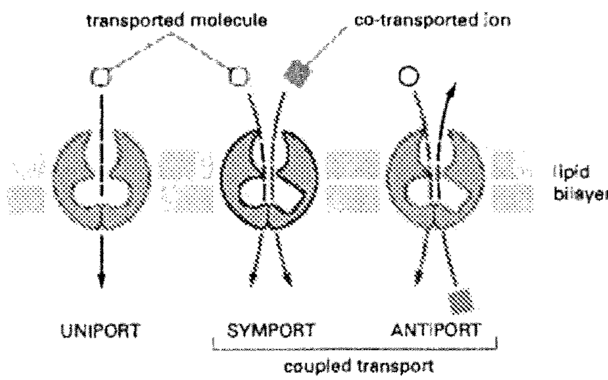

Figure 11–8 Three types of carrier-mediated transport. The schematic diagram shows carrier proteins functioning as uniports, symports, and antiports.

Although the molecular details are unknown, carrier proteins are thought to transfer the solute across the lipid bilayer by undergoing reversible conformational changes that alternately expose the solute binding site first on one side of the membrane and then on the other. A schematic model of how such a carrier protein might operate is shown in Figure 11–9. Because carriers are now known to be multipass transmembrane proteins, it is highly unlikely that they ever tumble in the membrane or shuttle back and forth across the lipid bilayer as was once believed.

As we discuss below, it requires only a relatively minor modification of the model shown in Figure 11–9 to link the carrier protein to a source of energy (such as ATP hydrolysis [see Figure 11–11] or an ion gradient) in order to pump a solute uphill against its electrochemical gradient. In fact, comparison of some bacterial carrier proteins with mammalian ones supports the idea that there need be little difference in molecular design between carrier proteins that mediate active transport and those that operate passively. Some carriers that in bacteria use the energy stored in the H^+ gradient across the bacterial plasma membrane to drive the active uptake of various sugars are structurally similar to the passive glucose carriers of animal cells. This suggests an evolutionary relationship between these carrier proteins; and given the importance of sugars as an energy source, it would not be surprising if this superfamily of sugar carriers were an ancient one.

We begin our discussion of active transport by considering a carrier protein that plays a crucial part in generating and maintaining the Na^+ and K^+ gradients across the plasma membrane of animal cells.

The Plasma Membrane Na^+-K^+ Pump Is an ATPase [7]

The concentration of K^+ is typically 10 to 20 times higher inside cells than outside, whereas the reverse is true of Na^+ (see Table 11–1, p. 508). These concentration differences are maintained by a **Na^+-K^+ pump** that is found in the plasma membrane of virtually all animal cells. The pump operates as an antiporter, actively pumping Na^+ out of the cell against its steep electrochemical gradient and

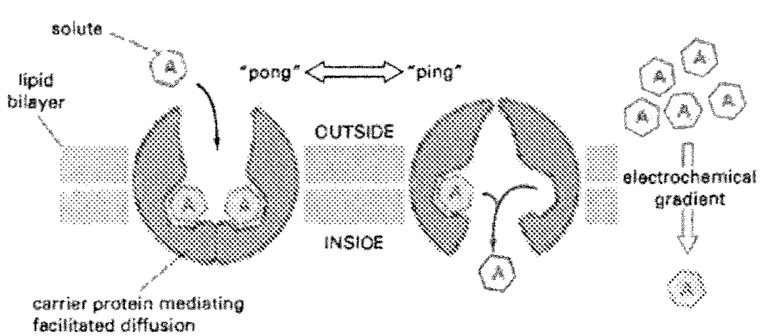

Figure 11–9 A hypothetical model showing how a conformational change in a carrier protein could mediate the facilitated diffusion of a solute. The carrier protein shown can exist in two conformational states: in state "pong" the binding sites for solute A are exposed on the outside of the bilayer; in state "ping" the same sites are exposed on the other side of the bilayer. The transition between the two states is proposed to occur randomly and to be completely reversible. Therefore, if the concentration of A is higher on the outside of the bilayer, more A will bind to the carrier protein in the pong conformation than in the ping conformation, and there will be a net transport of A down its electrochemical gradient.

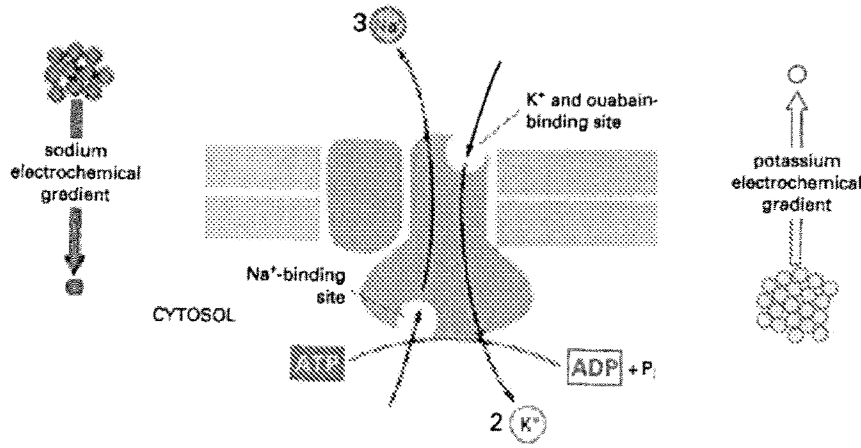

Figure 11–10 The Na^+-K^+ ATPase. This carrier protein actively pumps Na^+ out of and K^+ into a cell against their electrochemical gradients. For every molecule of ATP hydrolyzed inside the cell, three Na^+ are pumped out and two K^+ are pumped in. The specific pump inhibitor ouabain and K^+ compete for the same site on the external side of the ATPase.

pumping K^+ in. As explained below, the Na^+ gradient produced by the pump regulates cell volume through its osmotic effects and is also exploited to drive transport of sugars and amino acids into the cell. Almost one-third of the energy requirement of a typical animal cell is consumed in fueling this pump; in electrically active nerve cells, which, as we shall see, are repeatedly gaining small amounts of Na^+ and losing small amounts of K^+ during the propagation of nerve impulses, this figure approaches two-thirds of the cell's energy requirement.

A major advance in understanding the Na^+-K^+ pump came with the discovery in 1957 of an enzyme that hydrolyzes ATP to ADP and phosphate and requires Na^+ and K^+ for maximal activity. An important clue linking this **Na^+-K^+ ATPase** with the Na^+-K^+ pump was the observation that a known inhibitor of the pump, *ouabain*, also inhibits the ATPase. But the crucial evidence that ATP hydrolysis provides the energy for driving the pump came from studies of resealed red blood cell ghosts, in which the concentrations of ions, ATP, and drugs on either side of the membrane could be varied and the effects on ion transport and ATP hydrolysis observed. It was found that (1) the transport of Na^+ and K^+ is tightly coupled to ATP hydrolysis, so that one cannot occur without the other; (2) ion transport and ATP hydrolysis can occur only when Na^+ and ATP are present inside the ghosts and K^+ is present on the outside; (3) ouabain is inhibitory only when present outside the ghosts, where it competes for the K^+-binding site; and (4) for every molecule of ATP hydrolyzed (100 ATP molecules can be hydrolyzed by each ATPase molecule each second), three Na^+ ions are pumped out and two K^+ ions are pumped in (Figure 11–10).

Although these experiments provided compelling evidence that ATP supplies the energy for pumping Na^+ and K^+ ions across the plasma membrane, they did not explain how ATP hydrolysis is coupled to ion transport. A partial explanation was provided by the finding that, during the pumping cycle, the terminal phosphate group of the ATP is transferred to an aspartic acid residue of the ATPase and is subsequently removed, as explained in Figure 11–11.

The Na^+-K^+ pump in red blood cell ghosts can be driven in reverse to produce ATP: when the Na^+ and K^+ gradients are experimentally increased to such an extent that the energy stored in their electrochemical gradients is greater than the chemical energy of ATP hydrolysis, these ions move down their electrochemical gradients and ATP is synthesized from ADP and phosphate by the Na^+-K^+ ATPase. Thus the phosphorylated form of the ATPase (step 2 in Figure 11–11) can relax either by donating its phosphate to ADP (step 2 to step 1) or by changing its conformation (step 2 to step 3). Whether the overall change in free energy is used to synthesize ATP or to pump Na^+ out of the ghost depends on the relative concentrations of ATP, ADP, and phosphate and on the electrochemical gradients for Na^+ and K^+.

The Na^+-K^+ ATPase has been purified and found to consist of a large, multipass, transmembrane catalytic subunit (about 1000 amino acids long) and

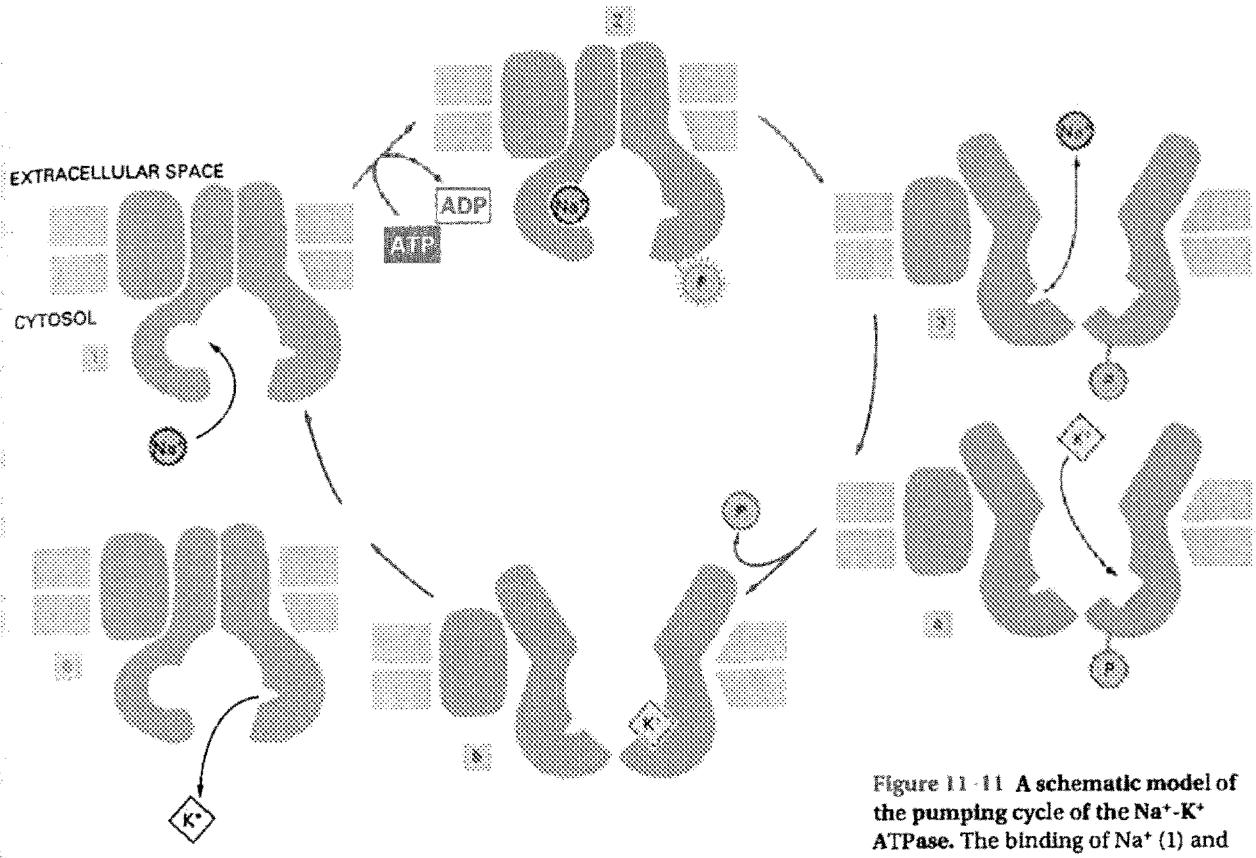

Figure 11–11 **A schematic model of the pumping cycle of the Na^+-K^+ ATPase.** The binding of Na^+ (1) and the subsequent phosphorylation by ATP of the cytoplasmic face of the ATPase (2) induce the protein to undergo a conformational change that transfers the Na^+ across the membrane and releases it on the outside (3). Then the binding of K^+ on the extracellular surface (4) and the subsequent dephosphorylation (5) return the protein to its original conformation, which transfers the K^+ across the membrane and releases it into the cytosol (6). These changes in conformation are analogous to the ping⇌pong transitions shown in Figure 11–9 except that here the Na^+-dependent phosphorylation and the K^+-dependent dephosphorylation of the protein cause the conformational transitions to occur in an orderly manner, enabling the protein to do useful work. Although for simplicity only one Na^+- and one K^+-binding site are shown, in the real pump there are thought to be three Na^+- and two K^+-binding sites. Moreover, although the ATPase is shown as alternating between two conformational states, there is evidence that it goes through a more complex series of conformational changes during the actual pumping cycle.

an associated smaller, single-pass glycoprotein. The former has binding sites for Na^+ and ATP on its cytoplasmic surface and a binding site for K^+ on its external surface, and is reversibly phosphorylated and dephosphorylated during the pumping cycle. The function of the glycoprotein is uncertain, except that it is required for the intracellular transport of the catalytic subunit to the plasma membrane. A functional Na^+-K^+ pump can be reconstituted from the purified complex: the ATPase is solubilized in detergent, purified, and mixed with appropriate phospholipids. When the detergent is removed, membrane vesicles are formed that pump Na^+ and K^+ in opposite directions in the presence of ATP (see Figure 10–22).

The Na^+-K^+ ATPase Is Required to Maintain Osmotic Balance and Stabilize Cell Volume [8]

Since the Na^+-K^+ ATPase drives three positively charged ions out of the cell for every two it pumps in, it is *electrogenic;* that is, it drives a net current across the membrane, tending to create an electrical potential, with the inside negative relative to the outside. This effect of the pump, however, seldom contributes more than 10% to the membrane potential. The remaining 90%, as we shall see later, depends on the pump only indirectly.

On the other hand, the Na^+-K^+ ATPase does have a direct role in regulating cell volume: it controls the solute concentration inside the cell, thereby regulating the osmotic forces that can make a cell swell or shrink (Figure 11–12). As explained in Panel 11–1, cells contain a high concentration of solutes, including numerous negatively charged organic molecules that are confined inside the cell (the so-called *fixed anions*) and their accompanying cations that are required for charge balance, and this creates a large osmotic gradient that tends to "pull" water into the cell. For animal cells this effect is counteracted by an opposite osmotic gradient due to a high concentration of inorganic ions—chiefly Na^+ and Cl^-—in the extracellular fluid. The Na^+-K^+ ATPase maintains osmotic balance by

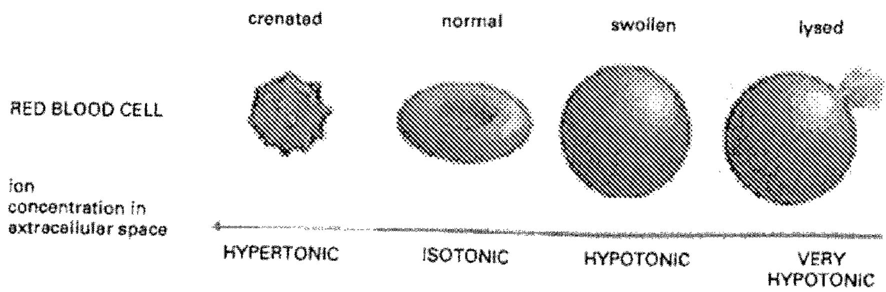

Figure 11–12 **Response of a human red blood cell to changes in osmolarity (also called *tonicity*) of the extracellular fluid.** Because the plasma membrane is freely permeable to water, water will move into or out of cells down its concentration gradient, a process called *osmosis*. If cells are placed in a *hypotonic solution* (i.e., a solution having a low solute concentration and therefore a high water concentration), there will be a net movement of water into the cells, causing them to swell and burst (lyse). Conversely, if cells are placed in a *hypertonic solution*, they will shrink.

pumping out the Na^+ that leaks in down its steep electrochemical gradient; the Cl^- is kept out by the membrane potential.

The importance of the Na^+-K^+ ATPase in controlling cell volume is indicated by the observation that many animal cells swell, and sometimes burst, if they are treated with ouabain, which inhibits the Na^+-K^+ ATPase. There are, of course, other ways for a cell to cope with its osmotic problems. Plant cells and many bacteria are prevented from bursting by the semirigid cell wall that surrounds their plasma membrane; in amoebae the excess water that flows in osmotically is collected in contractile vacuoles, which periodically discharge their contents to the exterior (see Panel 11–1). But for most animal cells, the Na^+-K^+ ATPase is crucial.

Some Ca^{2+} Pumps Are Also Membrane-bound ATPases [9]

Eucaryotic cells maintain very low concentrations of free Ca^{2+} in their cytosol ($\sim 10^{-7}$ M) in the face of very much higher extracellular Ca^{2+} concentrations ($\sim 10^{-3}$ M). Even a small influx of Ca^{2+} significantly increases the concentration of free Ca^{2+} in the cytosol, and the flow of Ca^{2+} down its steep concentration gradient in response to extracellular signals is one means of transmitting these signals rapidly across the plasma membrane. The maintenance of a steep Ca^{2+} gradient is therefore important to the cell. The Ca^{2+} gradient is in part maintained by Ca^{2+} pumps in the plasma membrane that actively transport Ca^{2+} out of the cell. One of these is an ATPase, while the other is an antiporter that is driven by the Na^+ electrochemical gradient.

The best-understood Ca^{2+} pump is a membrane-bound ATPase in the *sarcoplasmic reticulum* of muscle cells. The sarcoplasmic reticulum—a specialized type of endoplasmic reticulum—forms a network of tubular sacs in the cytoplasm of muscle cells and serves as an intracellular store of Ca^{2+}. (When an action potential depolarizes the muscle cell membrane, Ca^{2+} is released from the sarcoplasmic reticulum into the cytosol, stimulating the muscle to contract, as discussed in Chapter 16.) The Ca^{2+} pump, which accounts for about 90% of the membrane protein of the organelle, is responsible for pumping Ca^{2+} from the cytosol into the sarcoplasmic reticulum. (The endoplasmic reticulum of nonmuscle cells contains a similar Ca^{2+} ATPase, but in smaller quantities, so that it is harder to purify.)

The Ca^{2+} ATPase can be analyzed biochemically by the same methods as the Na^+-K^+ ATPase and is found to function in a closely similar way. DNA sequencing studies show, in fact, that the Na^+-K^+ ATPase and Ca^{2+} ATPases are homologous proteins. In each case the large catalytic subunit exists in multiple isoforms, is thought to have about 10 putative membrane-spanning α helices, and is phosphorylated and dephosphorylated during the pumping cycle.

Membrane-bound Enzymes That Synthesize ATP Are Transport ATPases Working in Reverse [10]

The plasma membrane of bacteria, the inner membrane of mitochondria, and the thylakoid membrane of chloroplasts all contain an enzyme that is analogous